Simulated Microgravity Induces SOST/Sclerostin upregulation in osteocytes

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ABSTRACT

Osteocytes are theorized to be the mechanosensors and transducers of mechanical forces in bone, yet the biological mechanism of this action remains elusive. Recent evidence suggests that SOST/Sclerostin is an important regulator of mechano-transduction.

To investigate the molecular mechanisms of SOST/Sclerostin regulation under in vitro and ex-vivo unloading we used the NASA Rotating Wall Vessel (RWV) Bioreactor. For in-vitro experiments, MLOY-4 osteocytic cells were seeded at a concentration of 250,000 cells onto 3D collagen scaffold (BD). Scaffolds (4 per condition) were either rotated in a vertical 50ml NASA/bioreactor vessel at 18 rpm (unloaded), cultured in a horizontal 50 ml NASA bioreactor vessel at 18 rpm (control for the sheared environment of vertical rotating vessel), or cultured in a static T-75 cm dish (static condition) for 7 days. For ex-vivo experiments, calvaria bones were harvested from 12-week old C57/Bl6 mice and sequentially digested with type I/II collagenase to remove periosteal osteoblasts. Calvaria halves (10 per condition) were then exposed to the same set of culture conditions described above.

Simulated unloading, as achieved in the NASA RWV, resulted in enlarged, round osteocytes, as assessed by H&E staining, that was reminiscent of prior reports of unloading causing loss of osteocyte morphology and dendritic network connectivity. Semiquantitative realtime qPCR and immunohistochemistry from both in-vitro and ex-vivo RWV experiments demonstrated a four-fold up-regulation of SOST/Sclerostin. Furthermore, mRNA of the transcriptional SOST enhancer Mef2C was upregulated 1.4 fold in ex-vivo calvaria subjected to unloading conditions of the NASA RWV, suggesting that Mef2C might be an important regulator of mechano-sensation. These findings are consistent with results from seven day hindlimb unloading experiments, C57/B6 females, conducted in our laboratory and validate the use of the NASA RWV as a tool to study osteocyte mechnotransduction.

BACKGROUND

- Osteocytes control osteoblast differentiation and activation via the Sclerostin-Wnt pathway
- Hindlimb unloading in mice upregulates SOST/Sclerostin expression in osteocytes (*Robling, et al., 2008*)
- SOST -/- mice are resistant to disuse-induced bone loss (Lin, et al., 2009)
- and postmenopausal woman stoke patients at six months showed elevated serum sclerostin levels (*Gaudio et.al, 2010*)
- Anti-sclerostin antibody treatments in clinical development pipeline

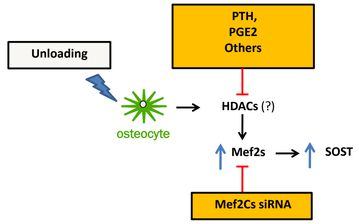


Figure 1: Proposed hypothesis for mechanical unloading regulation of SOST/Sclerostin

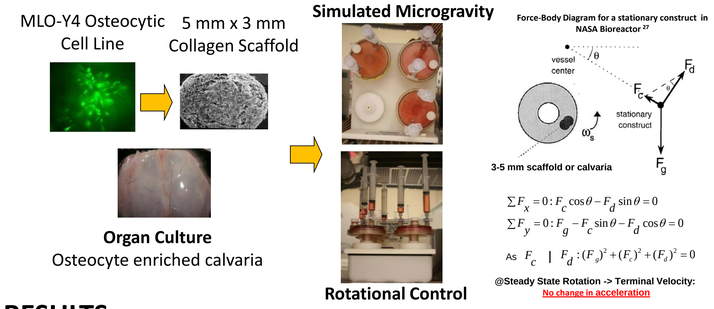
MATERIAL & METHODS

Cells: MLO-Y4 cells were grown to confluence, harvested and seeded onto 3D collagen scaffold (BD) at a concentration of 250,000 cells/scaffold.

Organ Culture: Calvaria were harvested from 3-to-5 day old mice and subjected to 6 collagenase digestions.

Simulated Microgavity & Rotational Control: Scaffolds (4 per condition) or calvaria were cultured were either rotated in a vertical 50ml NASA/bioreactor vessel at a speed of 18 rpm (simulated microgravity), cultured in a horizontal 50 ml NASA bioreactor vessel at a speed of 18 rpm (control for the increased sheared environment of rotating vessel), see Figure 2.

Static Control: Cells or calvaria were cultured in a T-75 cm dish (static condition)



RESULTS

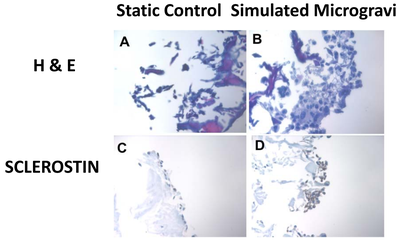


Figure 2: MLO-Y4 Osteocytic cells subjected to simulated microgravity: MLO-Y4 cells were grown on 3D collagen scaffold and subjected to static (A and C) or simulated microgravity (B and D) culture conditions. H&E staining (A and B) showed increase in cell proliferation in MLO-Y4 cultured under simulated microgravity condition (rotating bioreactor, panel B). Immunohistochemistry for Sclerostin (C and D) showed an increase in Sclerostin expression (black staining) in MLO-Y4 grown under simulated microgravity (panel D).

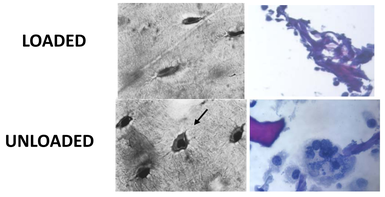


Figure 3: Unloading causes characteristic osteocyte morphologic changes: Osteocytes from immobilized rat (A and C, Krempien, 1976) and MLO-Y4 cultured on 3D collagen scaffold under simulated microgravity (B and D, H&E staining). Loaded osteocytes are spindle-shaped with small nuclei; unloaded osteocytes have enlarged round nuclei and cytoplasms.

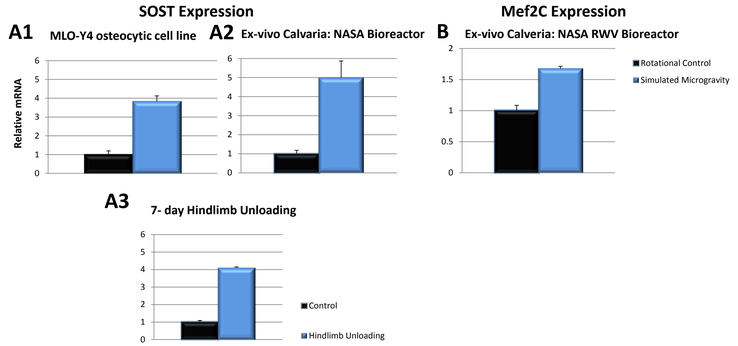


Figure 4: Simulated Microgravity increases SOST & Mef2C expression @ 7 days: Real-time qPCR for SOST and Mef2C mRNA in MLO-Y4 cells (A1) and calvaria (A2 and B) placed in simulated microgravity. Simulated microgravity induced a 4-to-6 fold increase in SOST and mRNA expression comparable to response in hindlimb unloaded mice. Results are expressed as relative RNA and are normalized by RPL13. Data are expressed as mean ± SD of triplicates. *: student unpaired two-tail t-test p<0.05

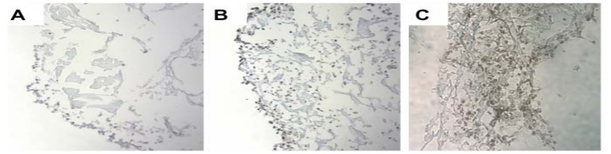


Figure 5: Sclerostin protein expression up-regulated @ 7 days: Sclerostin expression in MLO-Y4 cultured on 3D scaffold for 7 days under static (A), rotational control, (B), or simulated microgravity, (C).

CONCLUSIONS & FUTURE WORK

- Simulated Microgravity induces:
  - Osteocyte morphologic changes similar to immobilization unloading
  - Increase in SOST/Sclerostin & Mef2C expression relative to rotating control
- Future work is focused on validating results with additional osteocytic cell lines our lab is developing in preparation for a International Space Station flight experiment

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